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INFLUENCE OF CHRONIC AND REPEATED STRESS ON THE  
PITUITARY-ADRENAL SYSTEM AND BEHAVIOR

Final Technical Report

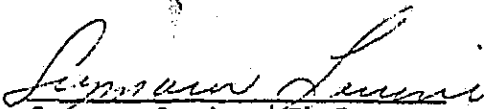
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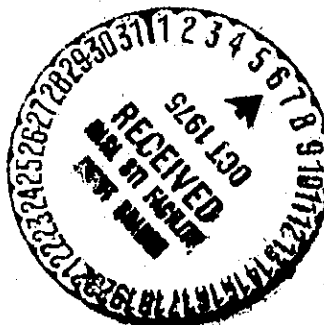
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During the period of time of the NASA grant we have made considerable progress in understanding the role of adrenal glucocorticoids and ACTH in behavior, and also the influence of various behavioral situations on the neuroendocrine regulation of the pituitary-adrenal system.

Initially our major emphasis was on the behavioral influence of ACTH and glucocorticoids on fear motivated behavior. Several years ago we demonstrated that ACTH facilitated passive avoidance. It was difficult to determine whether or not the administration of ACTH had an influence on the acquisition of a passive avoidance response or the extinction of the conflict behavior induced by the passive avoidance situation. In an intensive study (1) rats were trained and tested in a three-stage conflict procedure, the first stage of which was approach learning to obtain water. During the second stage passive avoidance was established by shocking animals for approaching the water and the third phase was an extinction phase during which time the animals were no longer shocked. The length of time it took for animals to emerge and approach the water was recorded. It was found that there was a significant increase in the response suppression, i.e. passive avoidance, following punishment for animals receiving ACTH at any one stage, whether ACTH was administered during approach training, avoidance training or test. Thus exogenous elevations of ACTH during any one of the three phases of passive avoidance procedure would produce some potentiation of the passive avoidance response. It would appear that ACTH therefore may have a multiplicity of effects; first, that it may weaken an approach response, strengthen an avoidance response, or increase fear during extinction. The fact that a single injection of ACTH during avoidance training markedly affects extinction clearly indicates an acquisition effect of ACTH on passive avoidance.

Having obtained an acquisition effect on passive avoidance, the question was asked if this acquisition effect is observable only in situations in which fear conditioning was involved or if a general property of ACTH is to influence the acquisition of any learned behavior.

A series of studies was initiated to determine whether the behavioral influence of these hormones could be extended to non-fear motivated situations. For the first experiment subjects were trained either under continual water reinforcement in an operant situation receiving ACTH or saline during training or extinction. ACTH during training significantly increased resistance to extinction of the response as measured by total responses per 10 minute extinction session. This was due primarily to an increase in the response by the ACTH groups early in extinction. Subsequently it was found, however, that ACTH also had a very marked influence on the acquisition of the operant behavior. It appears that we have demonstrated an effect of this polypeptide not only on extinction but also in the acquisition phase of the learning process. These studies demonstrated that the role of these hormones is not specifically involved in only fear conditioning situations.

Several years ago we reported that there were marked effects of pituitary-adrenal hormones on the Kamin effect (2, 3). Studies in our laboratory have indicated that both adrenalectomy and glucocorticoids implanted directly into the brain influence the retention of a previously learned avoidance response (Kamin effect). It was found that adrenalectomy almost completely abolished any retention of the previously learned avoidance behavior, whereas an implant of crystalline hydrocortisone into the median eminence of the hypothalamus led to significantly greater retention of the avoidance response. It is very tempting to interpret these data in terms of the influence of glucocorticoids

on memory.

In addition, other studies in this laboratory (4) have shown that there is a marked influence of glucocorticoids and pituitary peptides on the habituation of the startle response. Animals implanted with hydrocortisone show a much more rapid habituation, whereas adrenalectomized animals show retarded habituation. In other situations it does appear that adrenalectomy, i.e. the absence of adrenal corticoids, appears to make the animal far less capable of adapting to novel situations. Inasmuch as glucocorticoids are released primarily under conditions of novelty, it would appear that these hormones play a role in the general processes of habituation.

Although much of the research in this area has focused primarily on manipulating hormones and studying the effects on behavior, we have recently completed a series of experiments which have yielded intriguing findings concerning the effects of behavioral manipulations on hormonal output. These phenomena are seen in two different behavioral paradigms, (1) shock-induced fighting behavior, and (2) operant behavior. In several studies on aggression (5) we have compared three groups of animals in terms of their ACTH response following differing situations. The first was a traditional control group not treated in any way. The second was a group of animals shocked individually in the apparatus in which we usually test for fighting. The third group of animals was fought in pairs using a procedure now commonly known as shock induced aggression. Following the last fighting session blood samples were obtained and plasma corticoids and ACTH were determined. It was found that fighting significantly reduced the ACTH response when compared to shocked controls. This was a most surprising finding since when one observes animals being shocked individually or in pairs, intuitively it is easy to predict that the pairs of fighting animals are the most

stressed. However, physiologically it appears that aggressive behavior functions primarily to reduce internal physiological responses. We have hypothesized that this may be a general property of coping behavior. Using this paradigm we have recently investigated the influence of early experience on the ACTH response to shock and shock-induced fighting and find that whereas there is a significant difference in the nonhandled animals between shock and fighting, this difference does not occur in early handled rats. Thus, animals stimulated in infancy show markedly elevated ACTH but do not differentiate between the fighting and non-fighting situation.

We have also examined the effects of shock-induced fighting on catecholamine metabolism by the technique of intracerebral administration of tracer amounts of precursor norepinephrine. Four hours after an intracisternal injection of a small amount of dopamine the animals in each group were tested. Immediately after the testing session and at various times thereafter animals were sacrificed and catecholamine metabolism in three major brain areas was studied. At the end of the testing session, the fought animals did not show any significant changes in catecholamine metabolism in any brain area. Shock alone however evoked an increase in the rate of norepinephrine utilization in the brain stem. Investigators have found changes in brain norepinephrine levels and utilization rates as a function of exposure to electric shock. It is of interest therefore that paired exposure of rats to the same stimuli, thereby evoking fighting behavior, did not produce a change in catecholamine metabolism. However, during the hour subsequent to the termination of the testing session, the fought animals did show a marked increase in norepinephrine metabolism in both the brain stem and diencephalon. The shocked animals, on the other hand, showed a marked decrease in the rate of norepinephrine utilization and release in the

brain stem with no changes in the diencephalon or significant correlation between brain stem norepinephrine levels one hour after the fighting session and the frequency of fighting recorded during the session. Thus, following exposure to the same electric shock stimulations the shocked and fought animals showed patterns of brain norepinephrine metabolism directionally, temporally and anatomically different.

Several years ago we initiated a series of studies concerning changes in pituitary-adrenal activity following various manipulations of operant schedules (6). Primarily the question under investigation was whether one could observe increases in pituitary-adrenal activity during the extinction of an appetitive response. If these hormones are involved in extinction then one would expect that under normally occurring extinction procedures these hormones should be activated so that they can operate on the inhibitory mechanisms involved in extinction. Therefore, we conditioned animals to lever press for water in a continuous reinforcement schedule. Plasma corticosterone values were determined following reinforced sessions and after extinction. All animals were sampled under both conditions, using a balanced repeated measures design. In addition, pre-session or basal concentrations of plasma corticosterone were measured in at least one subgroup. The findings were that plasma corticosterone levels were significantly elevated as a function of extinction, while reinforced responding produced no changes compared to that of basal levels. The results showed, therefore, a major physiological change occurring as a consequence of extinction of an appetitive task. These data however could be interpreted in two ways: first, that the elevation occurring following extinction on a continuous reinforcement schedule is a consequence of frustrative non-reward; second, that activation of the pituitary-adrenal system occurs as a consequence

of the arousal invoked by changes in previous expectancies. Thus, organisms on continuous reinforcement, when extinguished, experience a marked change in expectancies concerning the reinforcement contingencies.

We have attempted to systematically investigate this latter hypothesis in a variety of ways. Incidental findings which have occurred as a consequence of these experiments have also been extremely interesting. Initially we studied animals which were on a variable interval (VI) schedule. During this schedule animals are reinforced approximately every 45 seconds with reinforcement occurring somewhere between 2 and 60 seconds during the session. After the animals were stabilized on this schedule they were shifted either to an extinction procedure or to continuous reinforcement (CRF). It was reasoned that a change from variable reinforcement to continuous reinforcement might indeed constitute a change in expectancies and lead to an increase in plasma corticoids. Much to our surprise we found, however, that whereas the VI animals when shifted to extinction did indeed show a marked elevation of corticoids, animals shifted from VI to CRF showed a significant suppression of corticoids. To follow up these findings we trained animals on a different operant paradigm, namely a fixed ratio (FR) reinforcement schedule. During this schedule animals are reinforced after 20 bar presses. Following stabilization animals were then shifted from a fixed ratio of 20 to either a CRF, FR10, FR40 or extinction. It was found that animals shifted from the FR of 20 to the higher FRs or extinction all showed elevations of plasma corticoids, whereas, once again, animals shifted to continuous reinforcement showed a suppression. These data would tend to argue against an expectancy notion and would indeed indicate that a change in the density of reinforcement to less reinforcement or no reinforcement constituted those sets of conditions which elevated plasma corticoids.



However, in a different experiment we feel that we have demonstrated that change in expectancies may be one set of conditions which influence activation of the neuroendocrine apparatus which controls pituitary-adrenal activity. In this experiment animals were trained on two different schedules. One group was trained on the VI schedule, the characteristic of this schedule being that the occurrence of reinforcement is unpredictable, and another group was trained on a fixed interval schedule, the characteristic being that reinforcement occurs every 45 seconds. During acquisition the FI animals tend to show lower operant rates since they are in effect anticipating the onset of the reinforcement pause before they begin to re-initiate operant responding. After the animals were stabilized on these procedures we then initiated a shift so that the VI animals were shifted to an FI schedule on a single session and the FI animals were shifted to a VI schedule. Thus the FI animals who previously had learned a temporal discrimination so that the onset of reinforcement was predictable, are now shifted to a nonpredictable situation, the opposite being true for the VI animals. Plasma corticoids were measured after the session in which the animals were shifted. VI animals shifted to FI showed no change in plasma corticoids. In contrast, it was found that FI animals shifted to VI, although the number of reinforcements achieved was essentially the same, did indeed show a marked and significant elevation in plasma corticoids, indicating that change of expectancy may be one of those factors which is involved in the activation of pituitary-adrenal activity. This hypothesis would tend to remove a lot of the ambiguity around the stress concept and permit specification of some of the environmental and cognitive conditions which may lead to an activation of the neuroendocrine mechanisms regulating pituitary-adrenal activity.

We have also been examining the role of certain limbic system structures

on the regulation of pituitary-adrenal function. In one study (7) male rats with bilateral lesions of the hippocampus or of the cortex overlaying the hippocampus were compared to unoperated rats on lever press for water reinforcement and extinction. The results showed that hippocampectomized animals lever pressed at a higher rate during extinction as expected. Plasma corticosterone levels were determined in the resting, nonstress stage and under water deprivation schedules and following ether anesthesia or exposure to a novel environment. In addition, measurements of plasma corticosterone were taken under a reinforced operant session or the first operant extinction session. The results indicated that hippocampal lesions cause no central deficit in pituitary-adrenal function when the animals are exposed to either ether or a novel environment. However, the hippocampectomized animals failed to exhibit the normal elevation of plasma corticosterone during the first extinction session, as seen now invariably in normal animals. These findings were interpreted as contradictory proposals that hippocampectomy increases the frustrative emotional response to unmet reward expectancies and supports theories related to the intentional process of hippocampal functions.

Other studies related to limbic system and pituitary-adrenal function have shown that septal lesions produce a deficit in pituitary-adrenal activity so that 45 days post-lesion of the septal area, rats show a significant decrement in the stress response and 90 days post-lesion the animals show significant adrenal atrophy and pituitary atrophy. Further examination of the role of the septum is required since the lesions in these studies were large and infringed upon the thalamus and other related brain structures.

In yet another study rhesus monkeys with surgical ablation of the amygdala were compared to normals in their response to restraint, that is, being placed

in a holding chair. It was found that amygdalectomized animals showed a marked reduction in the corticoid response to these conditions. This appeared in two experiments, one using previously well-trained animals, and another using naive animals. In contrast, amygdalectomized monkeys showed an exaggerated response to exogenously administered ACTH. It appears that the amygdalectomized animals have an endocrine system which is incapable of self-regulation and therefore unable to restore normal limits on steroid output. Therefore, in response to ACTH the amygdalectomized animals continue to rise even after two hours following the administration of ACTH while, by that time, the normal animals have leveled off and reached a plateau.

#### REFERENCES

1. Guth, S., J.P. Seward & S. Levine. Hormones and Behavior 2, 127-138, 1971.
2. Levine, S. In Endocrines and the Central Nervous System, R. Levine, ed., Williams & Wilkins, Baltimore, 1966. Pp. 280-291.
3. Levine, S. & F.R. Brush. Physiology & Behavior 2, 385-388, 1967.
4. Levine, S. Scientific American 224, 26-31, 1971.
5. Conner, R.L., J. Vernikos-Danellis & S. Levine. Nature 234, 564-566, 1971.
6. Coover, G.D., L. Goldman & S. Levine. Physiology & Behavior 6, 261-263, 1971.
7. Coover, G.D., L. Goldman & S. Levine. Physiology & Behavior 7, 727-732, 1971.